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DOI:
10.1152/ajpregu.00404.2016

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Document Version
Peer reviewed version

Citation for published version (Harvard):
Thomas, KN, van Rij, AM, Lucas, S & Cotter, JD 2017, 'Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls: lower-limb hot-water immersion in PAD', *AJP Regulatory Integrative and Comparative Physiology*, vol. 312, no. 3, pp. R281-R291. https://doi.org/10.1152/ajpregu.00404.2016

Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls

Running Title: Lower-limb hot-water immersion in PAD

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Abstract

Passive heat induces beneficial perfusion profiles, provides substantive cardiovascular strain and reduces blood pressure, thereby holding potential for healthy and cardiovascular disease populations. The aim of this study was to assess acute responses to passive heat via lower-limb hot-water immersion in patients with peripheral arterial disease (PAD) and healthy, elderly controls. Eleven patients with PAD (age 71±6 y, 7 male) and ten Controls (age 72±7 y, 8 male) underwent hot-water immersion (30 min waist-level immersion in 42.1±0.6°C water). Before, during and following immersion, brachial and popliteal artery diameter, blood flow and shear stress were assessed using duplex ultrasound. Lower-limb perfusion was measured also using venous occlusion plethysmography and near-infrared spectroscopy. During immersion, shear rate increased (p<0.0001) comparably between groups in the popliteal artery (Controls: +183±26%; PAD: +258±54%) and brachial artery (Controls: +117±24%; PAD: +107±32%). Lower-limb blood flow increased significantly in both groups, as measured from duplex ultrasound (>200%), plethysmography (>100%) and spectroscopy, while central and peripheral pulse wave velocity decreased in both groups. Mean arterial blood pressure was reduced by 22±9 mmHg (main effect p<0.0001, interaction p=0.60) during immersion, and remained 7±7 mmHg lower 3 h afterward. In PAD, popliteal shear profiles and claudication both compared favourably with those measured immediately following symptom-limited walking. A 30-min hot-water immersion is a practical means of delivering heat therapy to PAD patients and healthy, elderly individuals to induce appreciable

systemic (chronotropic and blood pressure lowering) and hemodynamic (upper and lower-limb perfusion and shear rate increases) responses.

Key Words: shear stress, passive heat, peripheral arterial disease, peripheral vascular disease, PVD, PAD, heat therapy, antegrade, shear rate, elderly

Introduction

Peripheral arterial disease (PAD) is a prevalent atherosclerotic disease, increasingly so with age for both men and women (1). It commonly manifests as intermittent claudication – walking-induced muscle pain that is the metabolic consequence of insufficient muscle perfusion during exercise. PAD is associated with faster functional decline (30) and increased cardiovascular morbidity and mortality (15, 22) than in those without PAD. As an alternative to conservative exercise therapy, heat therapy has been suggested to have potential in those with PAD (47), who are limited in their ability to perform traditional exercise for cardiovascular benefit. Indeed, heat has shown promise for its ability to reduce symptoms and improve several measures of lower-limb perfusion in PAD patients (44, 47). These results have not yet been replicated by others though, and the potential of heat as an acute stressor has not been fully characterized in this group. The acute responses will be important in understanding if the role of heat in providing clinical benefit is via an improvement in arterial function, via a downstream effector of increased perfusion or systemic hemodynamics, or via some other mechanism.

Recent evidence in healthy individuals has demonstrated that heat has potential to induce beneficial hemodynamic responses. Specifically, passive heat increases antegrade shear stress in the arteries of the upper (8, 51) and lower limb in healthy, young (11, 49) and older individuals (42), and in addition, *repetitive* hot-water immersion improves upper-limb artery function and structure (3, 5, 9, 34). Increased antegrade shear rate is thought to be the principle mechanism for exercise- (and likely heat-) induced improvements in arterial health (27, 36).

Most investigations on shear patterns and related adaptations have focused on the brachial artery, which is simple to assess and usually assumed to reflect global arterial responses and overall cardiovascular risk (10). Many interventions involve greater stress on the lower limbs, so upper-limb hemodynamics may not adequately represent those of the lower limbs, although are still of interest for characterizing the remote effects of the stimulus. And, atherosclerotic disease is far more prevalent in the lower limbs than upper limbs (32), so understanding the flow profiles in both upper-limb and lower-limb arteries – especially in those with disease – is important. Recently Romero et al. reported that lower-limb heating acutely improved macro- and microvascular function in healthy, elderly adults (42). Significant PAD serially narrows conduit artery cross-sectional area, or occludes an artery altogether, thereby increasing resistance to flow. Atherosclerosis in the arterial walls additionally reduces arterial compliance, so for these reasons the flow and shear profiles at rest already differ from those in healthy vessels (7), and the differential responses to heat in healthy and diseased vessels of elderly individuals have not been described.

Heat stress also causes several other significant physiological responses in humans, including increased core temperature, cutaneous blood flow, heart rate and cardiac output (13, 43, 53); all of which occur during an acute exercise bout (26). The acute hemodynamic and cardiovascular responses to lower-limb heating have not been fully characterised in individuals with PAD. Recent work by Neff and colleagues (35) demonstrated increased limb blood flow and reduced blood pressure in response to lower-limb heating (via a water-perfusion suit) in PAD patients. While they hypothesised that increased blood flow and arterial shear stress may mediate

improvements in vascular health in this group, they did not measure shear stress so this remains unquantified in PAD. We have previously used hot-water immersion to examine the hemodynamic responses (including shear stress) to heating in healthy, young participants, and to compare the flow profiles to those induced in response to exercise (49). Whether the responses observed in these healthy, young participants also occur in PAD, how they compare with those in healthy, elderly individuals is unknown. Also of importance, whether they translate into changes in function has not been determined. Understanding the acute hemodynamic and cardiovascular responses to heating in elderly individuals with and without arterial disease are of interest to inform potential long-term adaptations and to warrant pursuit of heat as a conditioning strategy in this patient population and others. To put the hemodynamic responses to heat in PAD into context, a comparison with the response to the current conservative therapy, walking, was included. A symptom-limited bout of treadmill walking was chosen as an ecologically-valid stimulus to characterize, as patients are seldom able to perform a traditional 30-min walk without stopping and resting. Finally, it seems reasonable to suggest that maximizing conductance of both major vascular beds within the leg (i.e., muscle and skin) may maximize the pressure gradient for perfusion, which might be achieved with a combined stimulus of local and whole-body heat stress applied in conjunction with localised exercise.

The aims of this study were therefore to assess in PAD patients and healthy, elderly controls: 1) The acute peripheral (upper- and lower-limb) hemodynamic effects of lower-limb hot-water immersion; 2) The acute systemic cardiovascular and thermal effects of lower-limb hot-water immersion; 3) Whether the responses differ

in PAD participants from those in healthy, elderly controls; and 4) if the responses are augmented by the addition of localised mild exercise during immersion. The final aim, 5) was to examine the responses to hot-water immersion relative to those from a symptom-limited bout of walking in PAD participants. We hypothesized that lower-limb hot-water immersion would induce significant increases in limb blood flow, shear stress and muscle perfusion in PAD and to a greater extent in healthy, elderly controls.

Materials and Methods

Experimental Design

Two cohorts were studied: PAD patients (PAD) and healthy, elderly controls. Each participant underwent two immersion sessions, as well as one exercise session for PAD only. One immersion was passive and one included mild intermittent exercise, to ascertain if the flow-increasing stimulus could be maximised by heat alone (i.e., passive immersion) or if the increased metabolic demand of exercised muscle would provide additional local and systemic effects (i.e., active immersion). Active immersion consisted of 3-min bouts of plantar flexion, performed at 0.5 Hz, at 10-min intervals (i.e., three bouts during immersion; Figure 1). The passive and active immersion sessions were administered in a randomised, cross-over fashion. Ethical approval was obtained from the Health and Disability Ethics Committee (14/STH/44), and the study conformed to the standards set by the Declaration of Helsinki.

Participant Characteristics

Inclusion criteria for PAD were: PAD confirmed by ankle-brachial index (ABI) of ≤ 0.7 at rest in at least one leg; mild to moderate claudication described corresponding to Fontaine Stage IIa to IIb (37); duplex ultrasound had been performed to confirm disease location and distribution; ≥ 50 years old, and; post-menopausal if female. Exclusion criteria for PAD were: Functioning bypass graft in situ; diabetes; previous occurrence of heat intolerance; unstable angina, or; myocardial infarction in the past 3 months. Inclusion criteria for controls were: ≥ 50 years old, no known history of PAD or other cardiovascular disease, resting ABI of ≥ 0.9 in both legs, no claudication, and post-menopausal if female. Exclusion criteria for controls were: known diabetes, PAD or cardiovascular disease, previous occurrence of heat intolerance. Participant demographics are shown in Table 1. Written consent was obtained and a questionnaire regarding health, medications and comorbidities was completed.

Experimental Protocol (see Figure 1)

Water immersion – Each participant completed the two immersion sessions, one to three weeks apart. The measurements taken during each were identical. Participants were asked to abstain from exercise on the day before each session, and to refrain from alcohol and caffeine for 12 h prior to testing. They were instructed to consume a standardised meal the evening prior to the session with ~ 10 mL water per kg body mass, and a standardised breakfast with 250 – 375 mL water between 6 and 7 am. Sessions began at or after 10 am and at the same time for each participant. Cessation of medications was not possible, but medication usage was continued as prescribed and recorded (Table 1). Sessions were performed in a

temperature-controlled environment at 24.4 ± 1.5 °C and ~40% relative humidity. Each immersion session began with a 20-min supine rest period, during which monitoring equipment was applied. Baseline measurements were then obtained (~1 h). For immersion, participants sat in a bath of hot water (maintained at 42.1 ± 0.6 °C) to the waist level for 30 min. Water temperature was checked continually and adjusted throughout the immersion. In the last 3 min of immersion, ultrasound measures (blood flow and shear rate) and plethysmography measures were repeated as described in each respective section below. Following immersion, measurements were repeated, beginning immediately and spanning ~45 min.

Treadmill exercise – A subgroup of PAD participants re-presented on a further occasion to perform a 3-min treadmill-walking test, at 3 km/h on a 10% incline. This is the standard exercise test used at this laboratory in the clinical diagnosis of PAD by exercise-induced reduction in ABI. Participants rated their claudication pain on a scale of 1 – 4 (2).

Measurements

Peripheral artery (brachial and popliteal) blood flow and shear rate were measured using ultrasound (Terason t3000, Teratech Corporation, Burlington, MA, USA) with a 10 MHz linear array transducer (bandwidth 5 – 12 MHz), by simultaneously recording a longitudinal section B-mode image and a spectral Doppler trace of blood velocity. Participants were supine during the brachial artery measurement, then adopted a lateral recumbent position (on the contralateral side to the leg being assessed) with the knee bent to 20-30° for the popliteal measurement. Measurements were made in the distal third of the upper arm and in the popliteal fossa, respectively; exact locations were marked and measured for

repeat tests. Ultrasound settings were optimised for each participant and reused for the repeat tests. The same certified and experienced vascular sonographer (KNT) performed all scans. Screen recording software (Camtasia Studio 8, TechSmith Corporation, Okemos, MI, USA) captured the screen in a video file for later offline analysis. Wall-tracking software (Cardiovascular Suite UE v 2.5, Quipu, Pisa, Italy) was used to determine diameter and velocity, and shear rate was calculated as: Shear rate = 4 * mean velocity / diameter (39, 41).

Blood flow was calculated as:

Flow = mean velocity * cross-sectional area,

Where mean velocity = peak envelope velocity / 2,

and cross-sectional area = π * (diameter/2)^2 (18, 29).

Test-retest reliability (coefficient of variation) for this operator using this software for measuring diameter and velocity was 0.4% and 2.1%, respectively (n = 10). The resting hemodynamics (D_{base}, velocity, shear rate and flow) of the brachial and popliteal arteries were assessed before and ~ 30 min after the intervention, from a 30 – 60 s recording period. In addition, popliteal and brachial hemodynamics were video-recorded for 30 s within the last 3 min of each immersion protocol. To obtain the popliteal artery measurements, the participant raised the knee to 20-30° and the sonographer reached around to the popliteal from a lateral window. During the treadmill exercise session the same variables were measured in the popliteal artery before and after exercise (recording began within 30 s of completing walking).

Venous occlusion plethysmography was used to measure calf blood flow via an indium-gallium strain gauge and plethysmograph (EC6, Hokanson, Bellevue WA, USA). Participants were supine with their leg elevated slightly above heart level. A

strain gauge was placed around the widest part of the calf and a cuff around the ipsilateral thigh was inflated to 50 mmHg to occlude venous outflow for 5 – 10 s. This was repeated 3 – 6 times until 3 reproducible traces were obtained. Data were transmitted to a computer via an analogue to digital converter (Powerlab/16SP, ADInstruments, Dunedin, New Zealand), and analysed later using Chart software (LabChart Pro v 7.2.5, ADInstruments). Limb volume changes were calculated from the steep linear portion of the plethysmographic trace following the inflation artifact, and the average of 3 measurements is presented. Calf inflow was assessed before, in the last 3 min of immersion, and within 5 min of completing the immersion.

Muscle oxygenation was measured using near-infrared spectroscopy (NIRS) on the posterior-medial calf (medial gastrocnemius muscle) of the leg under investigation, via probes housed in a light-shielding case attached to the skin with tape (NIRO-200; Hamamatsu Photonics KK; Hamamatsu, Japan). The NIRO-200 device measures changes in chromophore concentrations of oxy- and deoxyhemoglobin (ΔO_2Hb and ΔHHb) via the modified Beer-Lambert law, and provides depth-resolved measures of tissue O_2 saturation (total oxygenation index (TOI)) and tissue Hb content (i.e., relative value of the total hemoglobin normalised to the initial value, nTHI) using the Spatially Resolved Spectroscopy (SRS) method. The SRS-derived NIRS parameters limit contamination from superficial tissue via depth-resolved algorithmic methods, providing an index of targeted local tissue saturation (TOI) and perfusion (nTHI) (see Davies et al. (17) for recent review). A **3-min exercise test** was performed at baseline and post-immersion to assess the effect of thermal status on exercise-induced NIRS responses. Exercise consisted of supine

plantar flexion against a resistance band held at a standard length by a co-investigator (paced at 0.5 Hz using a metronome) and participants were asked to match the effort pre- and post-immersion. All NIRS data are presented as change values as is common practice to minimise the influence of variation between days and individuals, and in recognition of the limitations of the technique to accurately quantify absolute values due to factors that affect the absorbance and attenuation of input light (17).

Blood pressure (BP) was measured using finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). BP was validated intermittently throughout sessions using a non-invasive intermittent BP oscillometric measurement device, (BP+, Uscom, Sydney, Australia). **Heart rate** was obtained continuously using detection of the R wave of a three-lead electrocardiograph (lead II of ECG, ADInstruments). The LabChart ECG Analysis Add-On was used for quantifying the frequency of arrhythmias across a 5-min period before and within 10 min of completing immersion. **Arrhythmias** were defined by an abnormally-long compensatory pause following the ectopic beat. Baseline BP and heart rate data were collected over 5 min before each immersion, and end of immersion data consist of a 2-min average during the last 3 min of immersion. **Ambulatory BP** was measured using an ambulatory monitoring system (AMBP, Oscar 2, SunTech Medical Ltd, England) every 30 min for 3 h following each immersion session (beginning at the end of the supine measurements, ~1 h post-immersion), and for the same 3-h period on the day prior to testing, for comparison.

Pulse wave velocity (PWV) was measured as central (carotid-femoral) and peripheral (carotid-radial) PWV using a hand-held tonometer (SPT-301, Millar

Instruments, USA), following recommended guidelines (52). Pulse transmit times were calculated from the R-wave of the ECG to the foot of the pressure wave, and PWV was calculated as the anatomical distance / time (54) for each of the central and peripheral components, averaged over at least 20 cardiac cycles. The anatomical distance was calculated by subtracting the distance from the carotid location to the suprasternal notch and the radial or femoral site of measurement respectively.

Core body temperature was measured as the external auditory canal temperature (aural temperature, T_{au}) using a thermistor in a moulded plug (BetaTHERM 2.2K3A1B NTC thermistors, BetaTHERM, USA). The ear was covered with cotton wool taped in place to reduce the effects of ambient temperature. Participants' **ratings of body temperature and thermal discomfort** were noted using a 13- and 5-point scale, respectively (extended from (20), at 10-min intervals during the immersion.

Statistical Analysis

Participant characteristics were compared across groups using an unpaired t-test. A mixed-design two-way ANOVA was used where possible to examine effects of (i) passive and active hot-water immersion, (ii) hot-water immersion in PAD participants and Controls, and (iii) hot-water immersion and treadmill exercise in PAD participants. Post-hoc tests were performed where appropriate using the Holm-Šídák method with *p*-values corrected for multiple testing. However, some research questions were examined using paired t-tests because of the specific question (e.g., NIRS data represented as a change from baseline), following consultation with a biostatistician. For all analyses, $p \leq 0.05$ was considered statistically significant.

Results

Eleven PAD patients and ten Controls participated and completed both 30-min immersions except that one PAD participant undertook one immersion only (active). Seven PAD participants completed the exercise session. All PAD participants had an occlusion of the superficial femoral artery > 5 cm in length (n = 6) or more than one stenosis of > 75% (n = 5). No dependent measure showed a different response between passive and active immersions except for a larger inflow by plethysmography following active compared to passive immersion in PAD (+203% vs. +114%, $p = 0.04$), and no order effects were evident, so for simplicity in reporting and interpretation, all data are from the average of the two immersions for each participant.

Popliteal artery shear rate and blood flow

At baseline, total shear rate (i.e., net) was higher in PAD than in Controls ($p = 0.004$), while retrograde shear was lower ($p < 0.001$) and popliteal diameter was smaller ($p < 0.001$; Table 2). Popliteal antegrade shear rate increased ($p < 0.0001$) two- to three-fold **during immersion** in both groups, with PAD showing higher levels ($p = 0.01$) and a tendency for a larger increase (interaction: $p = 0.053$; Table 2 and Figure 2). Retrograde shear was absent in PAD throughout but in Controls decreased significantly during immersion (by 8 ± 5 /s, $p = 0.002$). Popliteal total shear rate increased ($p < 0.0001$) during immersion by a similar proportion in each group (interaction: $p = 0.11$), thereby remaining higher in PAD than Controls ($p = 0.003$).

At 30 min after immersion, antegrade shear rate remained above baseline levels ($p = 0.0002$) in both groups (change from baseline: Controls: +65%, PAD:

+43%), and higher in PAD than Controls (group: $p = 0.003$, interaction: $p = 0.07$, Figure 2).

Sample spectral Doppler traces obtained in the popliteal artery in a PAD and Control participant are illustrated in Figure 3. The velocity profile in the popliteal artery reflected lower resistance in response to immersion for both groups; the characteristics associated with this are described in the legend for Figure 3.

Blood flow in the popliteal artery was not different **at baseline**, but approximately tripled ($p < 0.0001$) **during immersion**, and tended to be higher in Controls than in PAD (group: $p = 0.07$, interaction: $p = 0.12$). Popliteal flow remained elevated ($p < 0.0001$) above baseline levels **at 30 min after immersion** (Controls: +72%, PAD: +71%) with no difference between groups ($p = 0.14$, interaction: $p = 0.51$; Figure 4). The diameter of the popliteal artery appeared to reduce during immersion in the Controls (by 9 ± 3%, $p = 0.007$) but not in PAD (-2 ± 3%, $p = 0.38$).

Brachial artery shear rate and blood flow

The brachial artery showed no difference in any hemodynamic variable between groups **at baseline**. Antegrade shear rate increased significantly **during immersion** ($p < 0.0001$; Table 2), and by an equivalent extent between groups (change from baseline: Controls: +107%, PAD: +117%; group: $p = 0.57$, interaction: $p = 0.68$). Retrograde shear rate was attenuated significantly ($p < 0.0001$) in each group during immersion, similarly so (by -12 ± 13 /s in Controls and -14 ± 8 /s in PAD; group: $p = 0.28$, interaction: $p = 0.70$). Thus, total brachial shear rate increased during water immersion in each group ($p < 0.0001$), and to a similar extent (group: $p = 0.50$, interaction: $p = 0.72$). **At 30 min after immersion**, antegrade shear rate

343 remained elevated (p = 0.0007) and to a similar extent in each group (change from
344 baseline: Controls: +88%, PAD: +104%; group: p = 0.53, interaction: p = 0.61).
345 Blood flow in the brachial artery was also increased significantly (p < 0.0001)
346 **during the immersion** (Controls: +282%, PAD: +176%), but overall was higher in
347 Controls (group: p = 0.03, interaction: p = 0.12). Flow remained above baseline **at 30**
348 **min after immersion** (p < 0.0001), to a greater extent in Controls (Controls: 139 ± 51
349 mL/min, PAD: 100 ± 31 mL/min; group: p = 0.04, interaction: p = 0.25). Arterial
350 diameter increased in each group during immersion (p = 0.0018) with no differential
351 effect (group: p = 0.26, interaction: p = 0.28; Table 2) and remained larger at 30 min
352 after immersion.

353 **Arterial inflow via plethysmography**
354 Inflow was similar between groups **at baseline** (Controls: 7.9 ± 2.4 vs. PAD:
355 10.4 ± 5.1 mL blood / 100 mL tissue / min; p = 0.18). Inflow increased **across**
356 **immersion** in each group (p < 0.0001) but more so in Controls (Controls: +380 ±
357 170% vs. PAD: +152 ± 104%; interaction: p = 0.0004). It remained ~234 and ~85%
358 elevated above baseline at **10 min after immersion** (Controls and PAD respectively).
359 Correlation between antegrade shear rate and inflow via plethysmography was only
360 moderate in Controls (R^2 = 0.49, p = 0.03); for remaining variables and all
361 comparisons in the PAD group, there were no significant correlations ($R^2 \leq 0.02$).

362 **Muscle oxygenation via NIRS**
363 Figure 5 illustrates the changes in O_2Hb, HHb, nTHI and TOI in response to
364 immersion in each group. The key findings were:
365 **At rest:** O_2Hb volume increased to a greater extent in Controls than in PAD
366 across immersion (difference between means: +151 μM·cm, 95% CI: 68 to 235

μM·cm, p = 0.002). The change in HHb volume in response to immersion did not differ significantly between groups (interaction: p = 0.14), nor did the increase in nTHI and TOI (nTHI: Controls: +0.16 a.u., PAD: +0.17 a.u.; interaction: p = 0.76; TOI: Controls: +6.7%, PAD: +5.8%; interaction: p = 0.56), indicating similar relative increases in local perfusion between the two groups.

In response to exercise:

A supine, 3-min bout of plantar flexion exercise in PAD elicited a greater drop in O_2Hb volume after immersion than before (by 98 μM·cm, 95% CI: -13 to -183 μM·cm, p = 0.03) and TOI (by 7.6%, 95% CI: -1 to -15%, p = 0.04), and a larger rise in HHb volume (p = 0.02), compared with before immersion. All parameters recovered from exercise at similar rates whether performed before or after immersion ($p \geq$ 0.08).

When comparing the response to exercise in PAD with Controls, not surprisingly, 3 min of plantar flexion exercise before immersion produced greater alterations from baseline in PAD than in Controls. O_2Hb volume dropped more in PAD (vs. Controls: -210 μM·cm, 95% CI: -357 to -64 μM·cm, p = 0.01), and HHb volume rose more (vs. Controls: +217 μM·cm, 95% CI: 55 to 380 μM·cm, p = 0.02). These changes and group differences were reflected in the TOI and nTHI derived measures of tissue saturation and regional blood flow (Figure 5c and d). Recovery of O_2Hb volume at 1 min post-exercise was heterogeneous in PAD and tended to be impaired more than in Controls (-137 μM·cm, 95% CI: -294 to 20 μM·cm, p = 0.08). The different responses between groups to 3 min of repeated contractions remained evident following immersion; O_2Hb and TOI declined despite increased nTHI in PAD, and failed to show complete recovery in PAD by 1 min of rest.

Systemic hemodynamics

At baseline, SBP, DBP, MAP and heart rate were not different between Controls and PAD (all $p \geq 0.1$). **During immersion**, heart rate increased ($p < 0.001$) similarly in both groups (interaction: $p > 0.35$). SBP, DBP and MAP were reduced by immersion (all $p \leq 0.001$) to a similar extent between PAD and Controls (Table 3; interaction effects: all $p \geq 0.16$) although overall SBP and MAP were higher in PAD (both $p = 0.04$). **At 30-min after immersion** SBP, DBP and MAP remained lower than baseline in both groups (all $p \leq 0.03$).

Ambulatory BP was recorded on both a control day and an immersion day for 9 Controls and 8 PAD. SBP was significantly ($p = 0.001$) lower following immersion in Controls and PAD (Controls: -14 ± 8 mmHg, PAD: - 5 ± 12 mmHg), with a tendency for more reduction in Controls (interaction: $p = 0.08$). DBP and MAP were also reduced significantly ($p = 0.047$ and $p = 0.003$ respectively) following immersion (DBP: Controls: -4 ± 7 mmHg, PAD: -6 ± 12 mmHg; MAP: Controls: -7 ± 7 mmHg, PAD: -6 ± 8 mmHg) with no differential effects between groups ($p \geq 0.64$ for all interaction and group effects).

At baseline, arrhythmias were present in 13 participants (6 Controls, 7 PAD). **During and following immersion**, one PAD participant showed an increase in arrhythmias on both occasions (9% to 38% and 6% to 79%), while one Control showed an increase following their first immersion (1% to 19%). A cardiologist reviewed the ECGs and diagnosed the arrhythmias as benign premature ventricular contractions. The remaining Controls and PAD all showed no obvious or consistent change in frequency of arrhythmias following immersion. One Control participant

414 experienced a vasovagal episode at the completion of the session on getting up from
415 supine rest. There were no adverse effects associated with this.

416 **Pulse wave velocity**

417 **At baseline**, neither central nor peripheral PWV differed between Controls
418 and PAD (central: Controls: 9.5 ± 1.7 m/s, PAD: 9.4 ± 2.1 m/s, $p = 0.87$; peripheral:
419 Controls: 8.0 ± 1.1 m/s, PAD: 7.2 ± 0.9 m/s, $p = 0.11$). **In response to immersion**,
420 central PWV decreased in each group ($p < 0.03$) by 1.0 ± 1.5 m/s in Controls and 0.5 ±
421 1.3 in PAD, and peripheral PWV decreased by 0.9 ± 1.2 m/s in Controls and 0.3 ± 0.7
422 m/s in PAD ($p = 0.01$). There was no differential effect evident between groups
423 (interaction: both $p \geq 0.15$). There were no significant correlations between baseline
424 PWV and change with immersion in either group for central or peripheral PWV (all p
425 ≥ 0.10, all $R^2 \leq 0.25$).

426 **Temperature**

427 The T_{au} increased +1.8 °C **with immersion** in both groups ($p < 0.01$, Table 3),
428 and recovered to lesser extent in PAD than in Controls ($p = 0.02$; remaining 1.4 ± 0.3
429 vs 1.0 ± 0.3 °C above baseline at 45 min after immersion). Perceived body
430 temperature was "hot" (i.e., 10 on the 13-point sensation scale) at the completion of
431 immersion for each group, and this was rated as comfortable to slightly
432 uncomfortable (Controls 1.6, PAD: 1.7 on the 5-point discomfort scale).

433 **Popliteal artery shear rate following 3-min treadmill exercise in PAD**

434 The 3-min treadmill-walking test was completed at the designated speed and
435 incline (distance ~150 m). All seven PAD patients reported claudication in the leg
436 studied, with an onset between 50 and 120 m, reaching moderate to intense pain (2-
437 3/4) at completion. Antegrade shear rate in the popliteal artery within 1 min of

completing exercise was elevated significantly from baseline, to 169 ± 81 /s (p = 0.02), with absent retrograde shear rate before and after exercise. The elevation in antegrade shear rate ($p < 0.0001$) caused by exercise or immersion was comparable (+112 /s and +102 /s respectively, condition: $p = 0.08$, interaction: $p = 0.79$). Similarly, average blood flow increased ($p = 0.0002$), to 91 ± 32 mL/min, a comparable increase to that seen during immersion (102 ± 46 mL/min, condition: p = 0.52, interaction: $p = 0.40$).

Discussion

A single bout of hot-water immersion induced shear stress patterns in the popliteal and brachial arteries of PAD participants and healthy, elderly controls that have been associated with beneficial adaptations (9, 34). This heat stress also induced positive chronotropy, increased lower-limb perfusion, and a marked lowering of blood pressure across at least the next 3 h. The regular repetition of this stress has potential to provide cardiovascular conditioning for PAD patients and other groups with limited access to exercise.

Lower-limb hemodynamics

At baseline

Despite no statistical differences in popliteal blood flow or arterial inflow between groups at rest, total shear rate was higher in PAD. This is likely explained by the smaller popliteal artery diameter in the PAD group, although may also reflect the different antegrade / retrograde shear components between groups. Unsurprisingly, hemodynamic differences between groups were revealed more obviously in response to 3 min of plantar flexion exercise (supine, before and after immersion), during which PAD participants showed a greater drop in tissue saturation, and impaired recovery of NIRS parameters at 1 min after exercise.

In response to immersion

Blood flow in the popliteal artery was increased by > 200% in PAD and Controls at the end of immersion, and remained elevated but to a lesser extent at 30 min post-immersion. Similarly, popliteal artery antegrade and total shear rate increased two- to three-fold during immersion in PAD. In healthy controls, popliteal

antegrade and total shear was also significantly elevated during and following immersion, but this tended to be to a lesser extent than in PAD (+180% during immersion); this relative disparity is again likely due to differences in vessel diameter between groups. The spectral Doppler blood velocity profiles changed during immersion in most PAD participants to exhibit continuous antegrade flow through the cardiac cycle (e.g., Figure 3b), which is interpreted clinically as an indicator of peripheral vasodilation and a lower resistance vascular bed downstream (38). Also reflecting lower resistance, a similar reduction in the retrograde component of the spectral waveform was demonstrated in most controls during immersion. The increased shear rates seen here is consistent with previous studies demonstrating increased antegrade shear in response to heating, but in the brachial artery in healthy individuals (8, 51), and also in the superficial femoral artery and common femoral artery in young individuals in response to mild (+0.5 °C) and moderate (+1.0 °C) passive heat stress respectively (11), although greater shear rates were seen at higher levels of passive heat. The lower-limb vessels are seldom studied, despite being more prone to disease than upper-limb arteries (32); to our knowledge this is the first study to describe this response of increased antegrade shear stress in diseased arteries. A doubling of popliteal blood flow in PAD has been reported from phase-contrast magnetic resonance imaging after 90 minutes of passive heat via a water-perfused suit (35); while this is encouraging that other methods of passive heating may also increase perfusion, the high conductive capacity of water immersion may be a more time-efficient and readily accessible method; at 30 min it had tripled blood flow in the present study. An unexpected finding was the reduction of the popliteal artery diameter in Controls. There is no obvious physiological

494 explanation for this; random or systematic error is possible, so further study is
495 needed to confirm or refute this finding.
496 In PAD participants, the antegrade shear rate elevation with immersion was
497 comparable to that achieved during a 3-min bout of treadmill walking, but
498 importantly, the immersion achieved this with no claudication, and is a stimulus that
499 can be applied for substantially longer than an exercise stimulus, for reasons of
500 tolerance. What remains unknown is whether the magnitude of the increased
501 antegrade shear stress demonstrated here is sufficient to induce beneficial vascular
502 adaptation in atherosclerotic arteries following repetition, or if they respond to a
503 shear stimulus in the same way at all. The relationship of transiently increased shear
504 stress to improvements in functional capacity in diseased vessels is yet to be
505 ascertained. There was considerable variation in the magnitude of the response
506 between individuals, which is understandable given the heterogeneity of the
507 disease, i.e., the variability in its location, distribution, severity and duration.
508 Two other measures of lower-limb perfusion demonstrated an increase in
509 both groups: NIRS-derived measures of tissue hemoglobin volume and saturation,
510 and plethysmography. The increased lower-limb perfusion likely comprises both
511 increased cutaneous and muscle blood flow, as local heating induces vasodilation of
512 both skin and muscle vasculature (23, 40). In PAD, the increased perfusion due to
513 immersion appeared to better support the metabolic demands of exercise,
514 evidenced by higher absolute post-exercise O_2Hb even in the face of a greater
515 exercise-induced drop. In Controls, the exercise bout before and after immersion
516 both produced much smaller perturbations in the NIRS-derived parameters of
517 oxygen extraction (i.e., HHb and TOI), which may indicate that perfusion of the

exercising muscles was better matched to the corresponding metabolic need, although a between group comparison is difficult with an exercise test of this nature. Overall a larger increase in both inflow and NIRS-derived measures of perfusion was seen in the muscle in Controls after immersion, indicative of a greater ability to respond with conduit and microvessel vasodilation to the heat stimulus. A limitation of using NIRS in this setting is baseline differences in tissue oxygenation were unable to be distinguished. Nevertheless, PAD participants demonstrated significant, relevant increases in all three measures of lower-limb perfusion.

In PAD, the adaptations to chronic obstruction and impaired flow result in anatomical and functional changes beyond commonly-used measures of vasodilatory function, such as beneficial modifications to muscle fibre characteristics (31), metabolism (25) as well as formation of collateral vessels (12). These or other responses may be adaptations to transiently increased blood flow e.g., angiogenesis and collateral vessel formation stimulated by hemodynamic forces (24). In patients with PAD in whom exercise to induce increased perfusion is not an appropriate option, the increased blood flow during and persisting after immersion is potentially the most clinically important finding of this study. However, whether this can translate to the long-term benefits and functional effects need to be examined. Overall, apart from understandable differences in the exercise response between groups, hot-water immersion functioned to a similar extent as a hemodynamic stressor in both groups. There were no significant differences between passive and active immersion for all but one dependent variable (inflow via plethysmography). On balance it appears that the addition of mild lower-limb exercise did not add to the hemodynamic strain induced by the immersion itself. The 3-min bouts were

perhaps of insufficient intensity or duration, or could have been different relative intensities between individuals, to reveal a group effect.

Upper-limb hemodynamics

The upper-limb responses were qualitatively very similar to those of the lower-limb: antegrade and total shear rate were increased and retrograde shear rate was attenuated by immersion, and by similar extents between groups. These findings are consistent with the data from Carter et al. (9), in which lower-limb heating acutely increased shear rate in the brachial artery, and this resulted in functional adaptations after repetition. The absolute shear rate and flow induced in the study by Carter et al. were approximately twice those presented here, but their participant demographic – healthy, young, active participants – may explain these differences. Furthermore, it is not currently known if a threshold exists for shear-mediated adaptations. The inclusion of the upper-limb hemodynamics provided insight into the general arterial responsiveness in non-atherosclerotic vessels (and those usually studied) to this form of stress, relative to diseased vessels within the same individual, as well as compared with healthy upper-limb vessels in Controls free from PAD. The upper-limb hemodynamic alterations seen with lower-limb hot-water immersion were similar between groups (and between upper and lower limbs), and thus highlight the systemic nature and wide-ranging applicability of the stressor.

Cardiovascular strain

Heart rate increased by > 30% during immersion in each group, i.e., within the range recommended for cardiac benefits from exercise training for an average-aged participant in this study (50-75% max heart rate (19) for a 72 year-old: 74 – 111

566 beats/min). Passive heat stress also has a beneficial inotropic effect on the heart (13)
567 (by virtue of a decreased MAP, reduced central blood volume and therefore
568 ventricular filling pressure), but preserved or elevated cardiac output. These effects
569 have been demonstrated in healthy individuals (4, 14) and in patients with CHF (46,
570 48). The lower-limb immersion protocol may therefore be useful for inducing an
571 increase in cardiac work by virtue of chronotropic and inotropic changes without the
572 concomitant increases in afterload usually experienced during exercise. It may
573 therefore be a gentler, more appropriate stressor for those with a high
574 cardiovascular disease burden. Accordingly, studies of repeated heat stress in
575 congestive heart failure patients have shown improvements in multiple parameters
576 of cardiac function (33, 46, 48). Of note, the induced thermal stress used in this
577 study had little effect on the prevalence arrhythmias.

578 Furthermore, the temperature of heart tissue, not work per se, provokes
579 upregulation of heat shock proteins (45), and this may be the case for other
580 adaptations as well, such as the induction of protection from ischemic reperfusion
581 injury (16, 24). Therefore, the significant aural temperature elevation, which
582 occurred to a similar extent in both elderly groups (almost 2 °C above baseline), is
583 important for the provision of strain for the cardiovascular system, and the local
584 effects on tissue and organs (e.g., heart). Measurement of temperature via auditory
585 canal thermistor provides an indirect index of core temperature; nevertheless, waist-
586 level hot-water immersion has been shown to increase oesophageal measurement
587 of core temperature to a comparable extent (6).

588 Alongside the chronotropic effect was a significant reduction in BP in both
589 groups. This hypotensive effect was greatest at the end of the immersion, with an

590 average reduction in MAP of ~22 mmHg. Hypotension persisted for several hours,
591 albeit measured in a subsample of participants, and was most consistent in controls.
592 A major portion of the health-related benefits of regular bouts of stress is
593 attributable to the recovery period itself, e.g., post-exercise hypotension is likely
594 more important in cardiovascular risk reduction than the small reduction in resting
595 blood pressure induced by exercise training (50). The full duration of the post-
596 immersion hypotensive effect has yet to be determined, but the implications of this
597 are particularly valuable in a PAD population, who are commonly hypertensive yet
598 commonly unable to exploit a post-exercise hypotensive effect. A hypotensive effect
599 of similar magnitude and duration has been demonstrated in PAD participants
600 undergoing passive heating via a water-perfused suit (35).
601 Passive heat stress has been shown to reduce PWV acutely, with the largest
602 effect seen in those with highest PWV at normothermic baseline (21). The effect of
603 immersion in this study was to reduce central and peripheral PWV in both groups,
604 with no obvious relationship to normothermic baseline PWV. A reduction in PWV
605 may afford a reduction in myocardial work and an increase in coronary perfusion
606 (28), again potentially beneficial in a high cardiovascular risk group.
607

608 *Perspectives and Significance*

609 This study has demonstrated acute hemodynamic, thermal and cardiovascular
610 responses to relatively brief immersion of the lower limbs in hot water; responses
611 that would be promising for cardiovascular conditioning in those less able to achieve
612 this by exercising. In particular, sustained increases in popliteal and brachial

antegrade shear rate were demonstrated, in elderly individuals with and without PAD. At least in healthy vessels, these shear stress profiles are known to stimulate functional then structural adaptation. Blood flow in the lower limbs of both groups was also increased by immersion. Qualitatively, the hemodynamic responses to immersion in those with and without arterial disease were similar, despite measures of perfusion increasing to a greater magnitude in non-diseased participants. The presence of PAD also did not appear to significantly alter the acute systemic cardiovascular response. The results of this study complement those from Neff et al. (35), together endorsing the further examination of lower-limb heating as a therapeutic approach for PAD patients, as has previously been suggested (44, 47), and for elderly individuals who cannot exercise for whatever reason. A natural progression of this work is to explore the clinical and functional outcomes of the repetition of this stressor as a therapeutic tool in PAD.

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Table and Figure Legends

Table 1

Participant demographics.

n, number; SD, standard deviation; BMI, body mass index; ABI, ankle-brachial index; PVR, pulse volume recording; IQR, interquartile range. * different from Controls ($p < 0.05$).

Table 2

Popliteal and brachial artery hemodynamic measures at baseline and in the last 3 min of immersion. SR, shear rate.

Data are mean ± SD for baseline and post values. Change scores are mean ± SE. † different from baseline ($p < 0.05$); * different from Controls ($p < 0.05$).

Table 3

Whole-body thermal and cardiovascular strain in response to 30-min hot-water immersions in PAD and Control participants.

Data are mean ± SD. Change scores (Δ) shown are percentages, apart from T_{au} which is shown in °C as indicated above. Baseline data are the average of the two baselines as there were no differences between sessions. Baseline data were averaged over 5 min, end-immersion data were averaged over 2 min within the last 3 min of immersion. Tau, aural temperature; MAP, mean arterial pressure; SBP, systolic blood

pressure; DBP, diastolic blood pressure; HR, heart rate. † different from baseline (p < 0.05). No significant difference between groups.

Figure 1

Schematic of experimental protocol for a) an immersion session (PAD and Control each performed two immersion sessions, one active and one passive), and b) the exercise session (for PAD only). PWV, pulse wave velocity; VOP, venous occlusion plethysmography; NIRS, near-infrared spectroscopy; BP, blood pressure. Hemodynamic assessments included diameter, blood flow and shear rate.

Figure 2

Popliteal artery total, antegrade and retrograde shear rate at baseline, during the last 3 min of immersion and 30-min post-immersion. † different from baseline (p < 0.05).

Figure 3

Sample spectral Doppler traces from the popliteal artery obtained from one control participant (left) and one PAD participant (right) at a) baseline, b) during the last 3 min of immersion and c) 30-min post-immersion. The x-axis represents time, the y-axis represents velocity, in cm/s. Note the different velocity scales. For the control participant, in a) this is a typical triphasic waveform of a normal, healthy peripheral artery, demonstrating moderate resistance, with a portion of the cardiac cycle demonstrating retrograde flow followed by a further antegrade component associated with good compliance. In b) the waveform is still triphasic, but a smaller proportion of flow is retrograde, and the peak systolic velocity has increased.

Similarly in c), there is a smaller retrograde component than in a). For the PAD participant, in a) this is a monophasic waveform demonstrating high resistance, with no flow seen for a significant portion of the cardiac cycle. In b) and c) the waveforms have become lower resistance with higher peak systolic velocity and antegrade flow throughout the cardiac cycle.

Figure 4

Popliteal artery blood flow at baseline, during the last 3 min of immersion and 30-min post-immersion in Controls and PAD. † different from baseline ($p < 0.05$).

Figure 5

Changes in muscle hemoglobin variables in response to lower-limb hot water immersion in Controls and PAD. a) O_2Hb, oxyhemoglobin response to immersion; b) HHb, deoxyhemoglobin response to immersion; c) nTHI, normalised tissue hemoglobin index; d) TOI, total oxygenation index. In each panel, Controls are shown on the left and PAD on the right. The grey background indicates pre-immersion and the white background indicates post-immersion. End of ex, end of 3-min plantar flexion exercise. † different from baseline ($p < 0.05$); ‡ different from pre-immersion exercise response ($p < 0.05$); * different from Controls ($p < 0.05$).

874 ***Table 1***

875

	Control	PAD
Sample size, n	10	11
Male, n	8	7
Age, years, mean (SD)	72 (7)	71 (6)
BMI, kg/m^2, mean (SD)	26 (3)	24 (5)
ABI for leg studied, mean (SD)	1.17 (0.11)	0.61 (0.11) *
PVR for leg studied, median (IQR)	Not performed	2 (2-3)
Medications		
Blood pressure control	1	8
Nitrates	0	1
Statins	1	7
Antiplatelet	1	6

876

877

878

879 ***Table 2***

880

	Controls			PAD		
Variable	**Baseline**	**Immersion**	**Δ, (%)**	**Baseline**	**Immersion**	**Δ, (%)**
Popliteal Artery						
Total SR (/s)	21 ± 9	89 ± 42 †	+366 ± 69	51 ± 28 *	152 ± 57 *†	+260 ± 54
Antegrade SR (/s)	32 ± 9	92 ± 40 †	+183 ± 26	51 ± 28 *	152 ± 57 *†	+258 ± 54
Retrograde SR (/s)	-12 ± 6	-4 ± 4 †	-67 ± 9	0 ± 0 *	0 ± 0	0 ± 0
Diameter (mm)	7.5 ± 1.2	6.8 ± 1.0 †	-9 ± 3	5.0 ± 0.6 *	4.8 ± 0.7 *	-2 ± 3
Flow (mL/min)	47 ± 14	150 ± 61 †	+229 ± 44	36 ± 23	102 ± 54 †	+226 ± 48
Brachial Artery						
Total SR (/s)	81 ± 49	205 ± 123 †	+189 ± 24	68 ± 28	177 ± 100 †	+166 ± 50
Antegrade SR (/s)	95 ± 40	207 ± 122 †	+117 ± 24	87 ± 26	181 ± 96 †	+107 ± 32
Retrograde SR (/s)	-14 ± 16	-2 ± 6 †	-125 ± 52	-19 ± 7	-5 ± 6 †	-74 ± 11
Diameter (mm)	4.9 ± 0.8	5.2 ± 0.7 †	+7 ± 3	4.6 ± 0.5	4.8 ± 0.6 †	+4 ± 2
Flow (mL/min)	57 ± 38	149 ± 43 †	+282 ± 79	37 ± 12	102 ± 46 *†	+176 ± 35

881

882 ***Table 3***

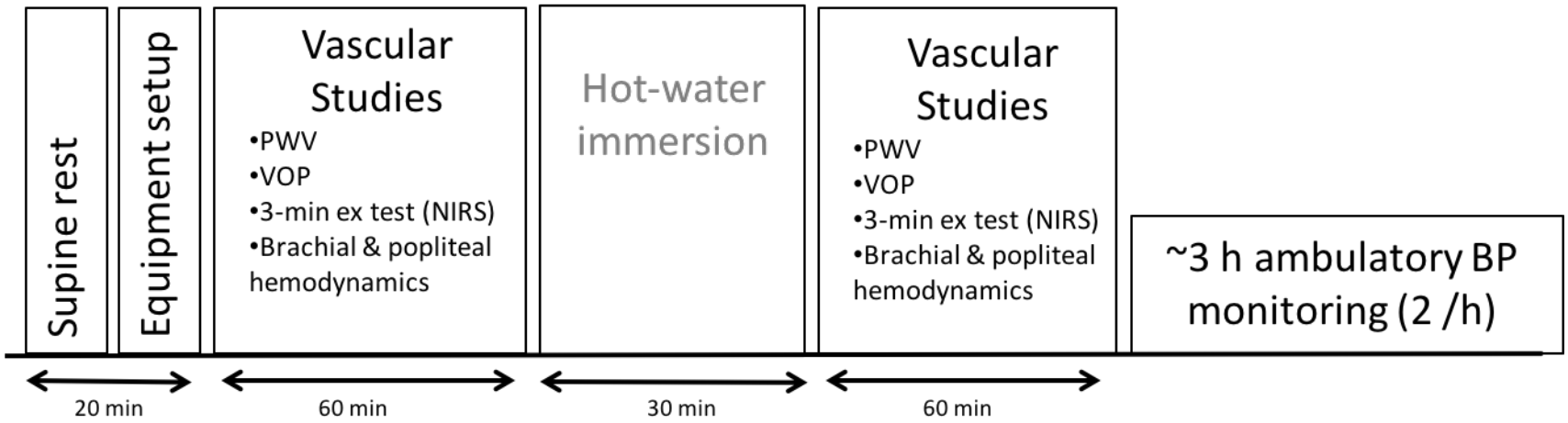
883

	Controls			PAD		
Variable	**Baseline**	**Immersion**	**Δ (%)**	**Baseline**	**Immersion**	**Δ (%)**
T_{au} (°C)	35.4 ± 0.4	37.2 ± 0.5 †	+1.8 ± 0.4 °C	35.1 ± 0.6	36.9 ± 0.4 †	+1.8 ± 0.3 °C
MAP (mmHg)	96 ± 7	73 ± 5 †	-23 ± 7	104 ± 15 *	83 ± 12 *†	-20 ± 8
SBP (mmHg)	144 ± 15	104 ± 7 †	-26 ± 8	158 ± 23 *	121 ± 20 *†	-23 ± 10
DBP (mmHg)	71 ± 5	57 ± 5 †	-20 ± 7	77 ± 13	64 ± 10 †	-16 ± 8
HR (beats/min)	62 ± 9	89 ± 17 †	+43 ± 21	59 ± 9	81 ± 13 †	+37 ± 16

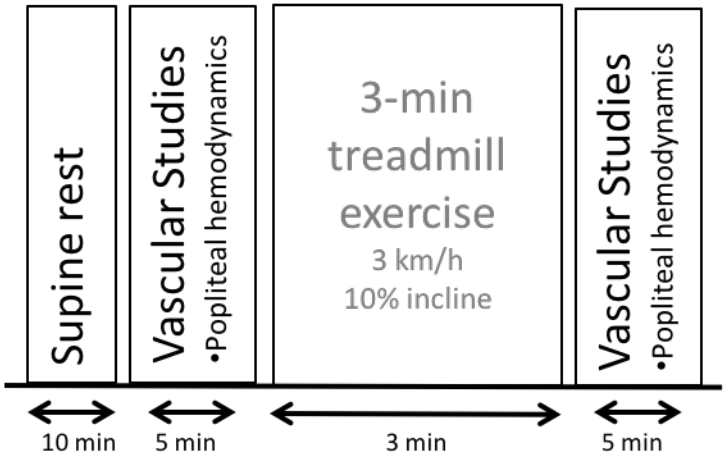
884

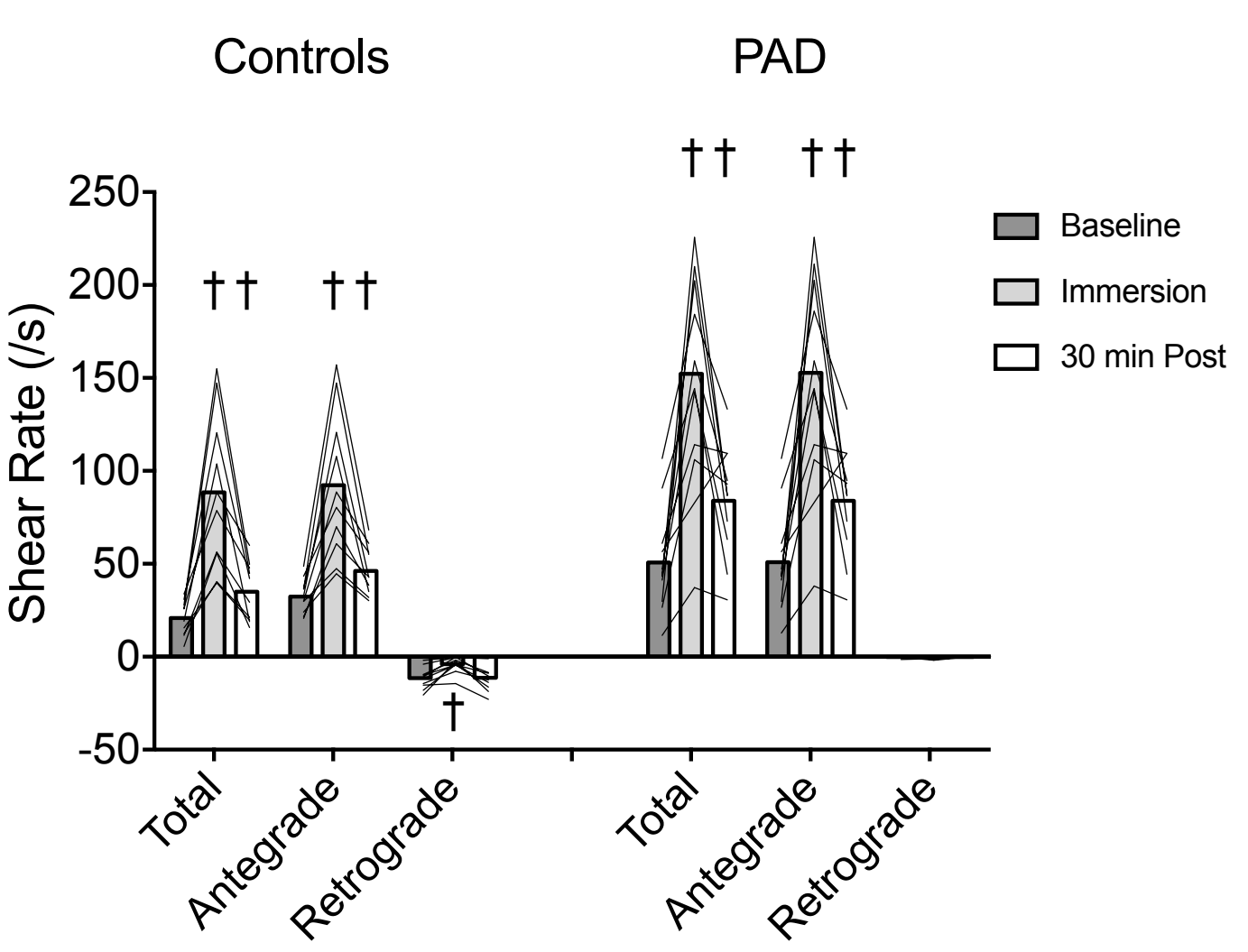
885

a) Water immersion

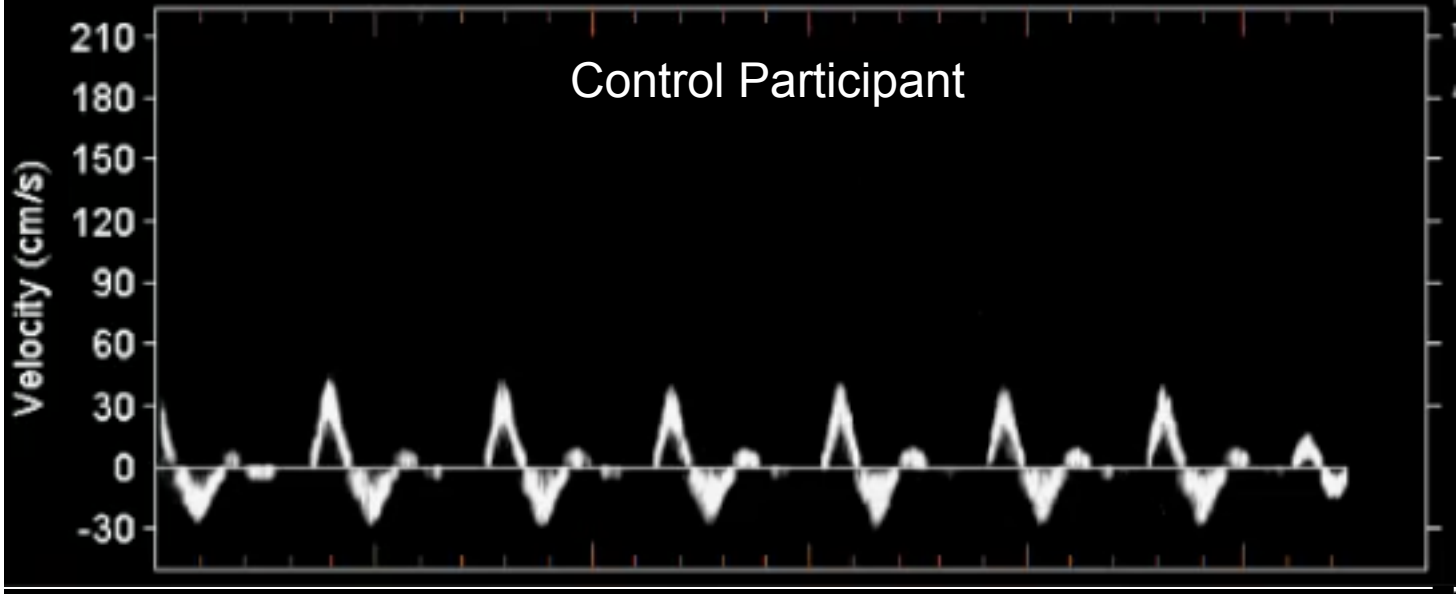


b) Treadmill exercise

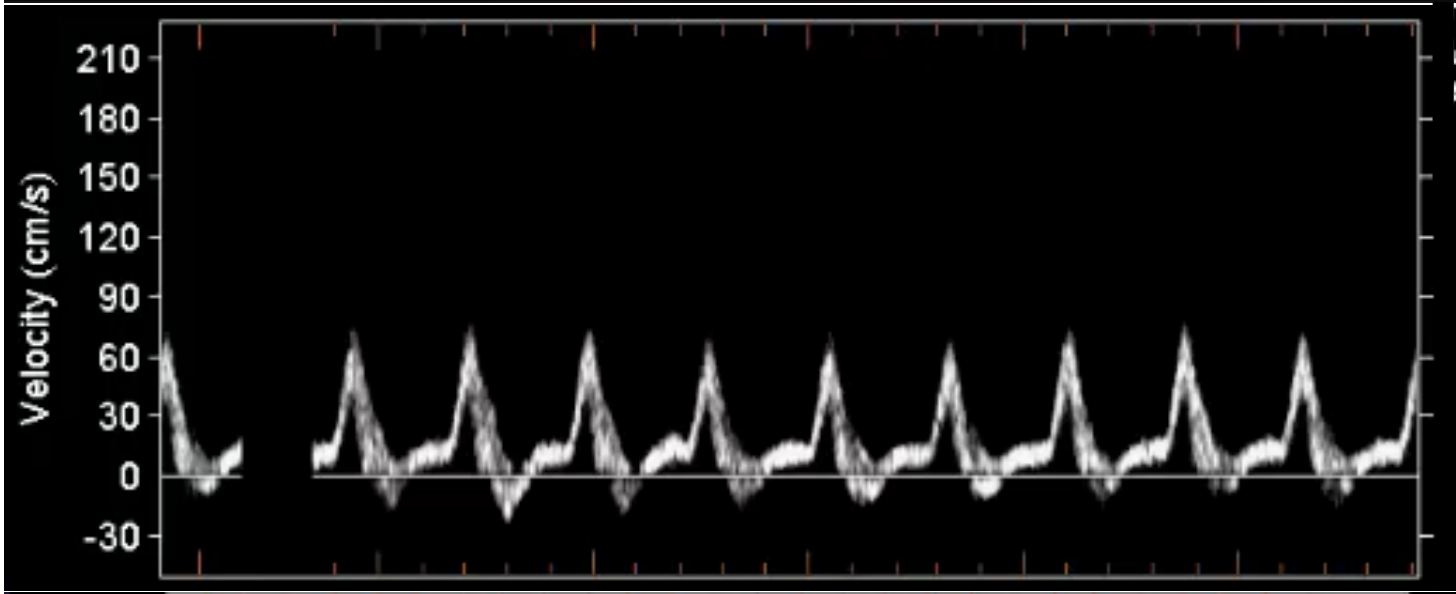



Controls
PAD
Shear Rate (/s)
250
200
150
100
50
0
-50
†† ††
†
†† ††
Baseline
Immersion
30 min Post
Total
Antegrade
Retrograde
Total
Antegrade
Retrograde

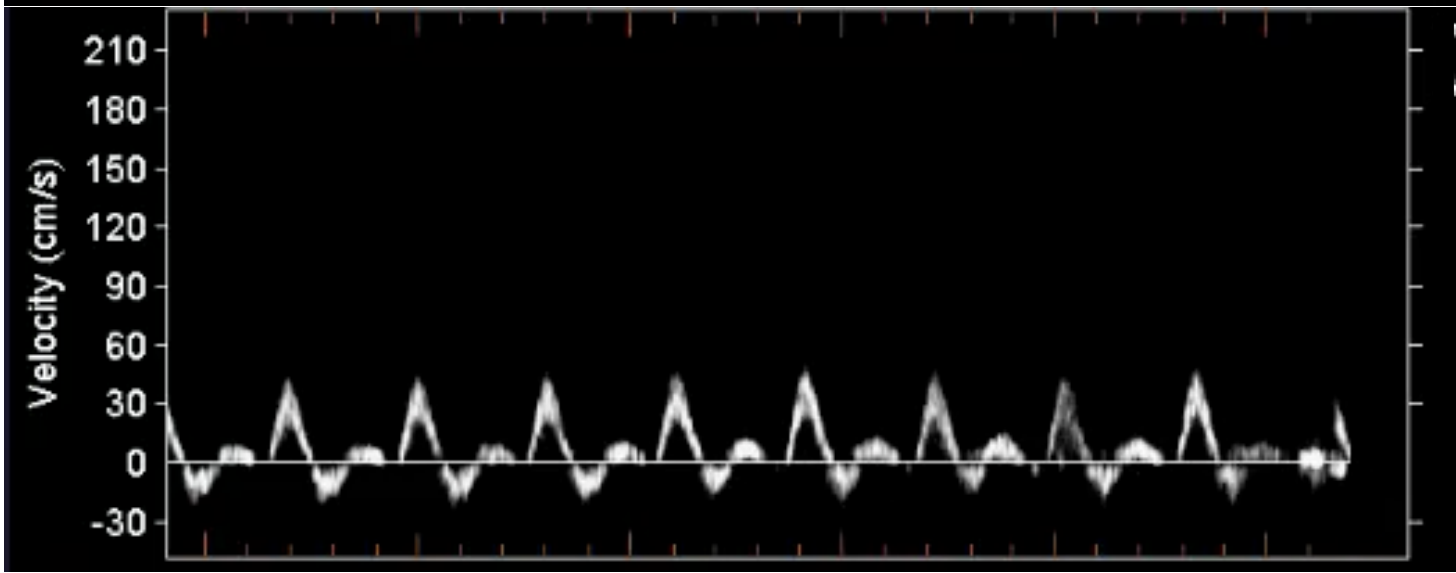
a) Baseline



b) Immersion



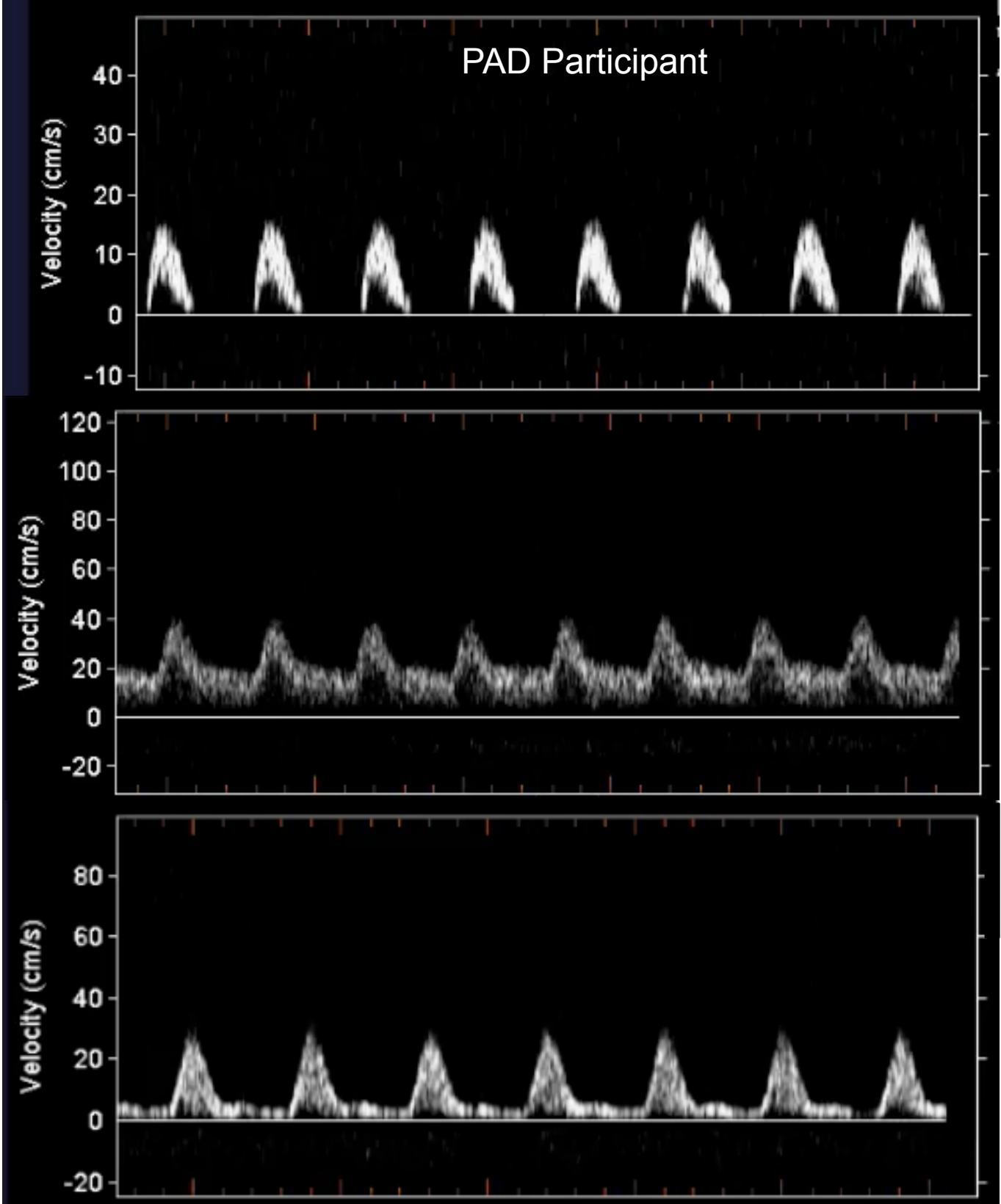
c) Post

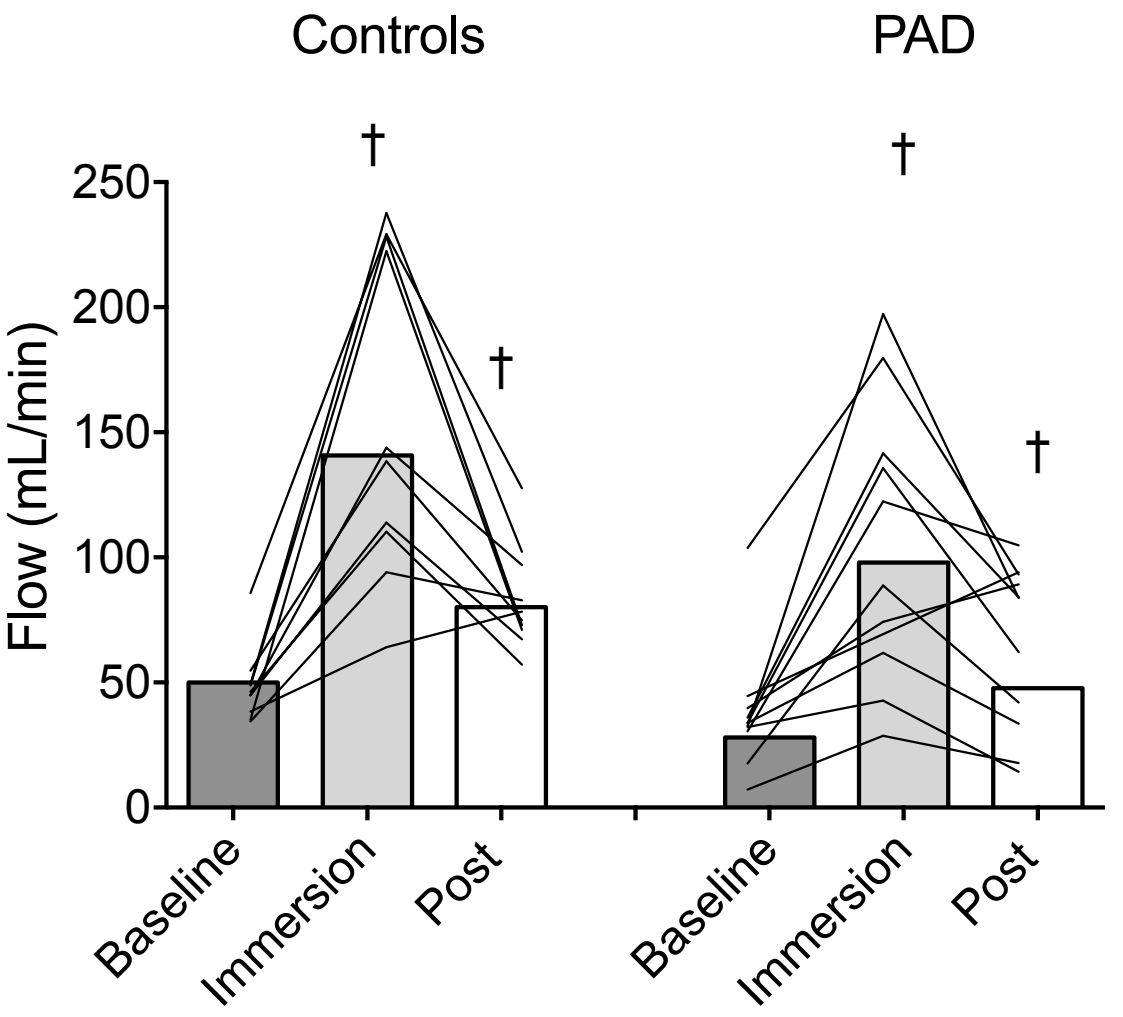


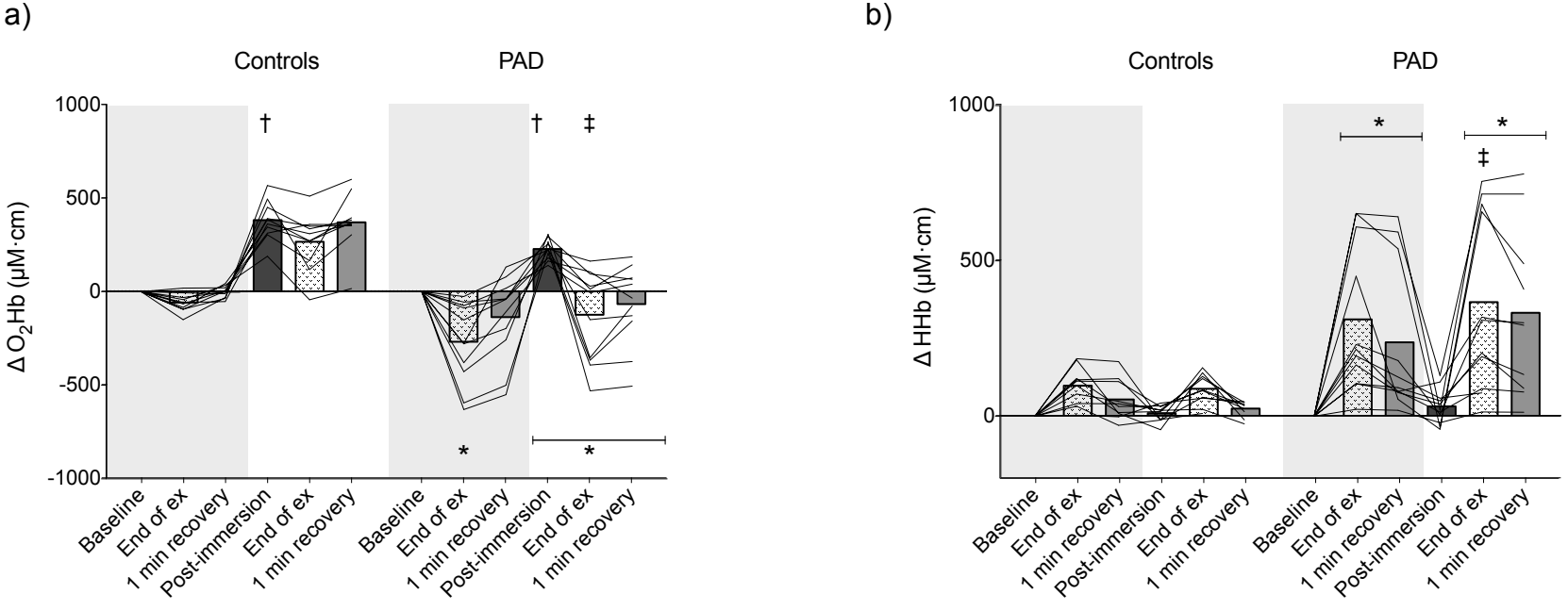
a) Baseline

b) Immersion

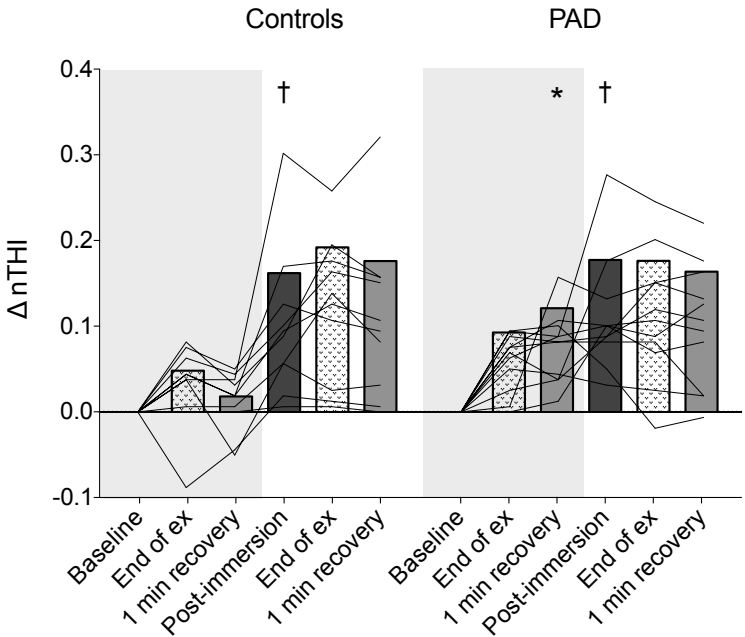
c) Post



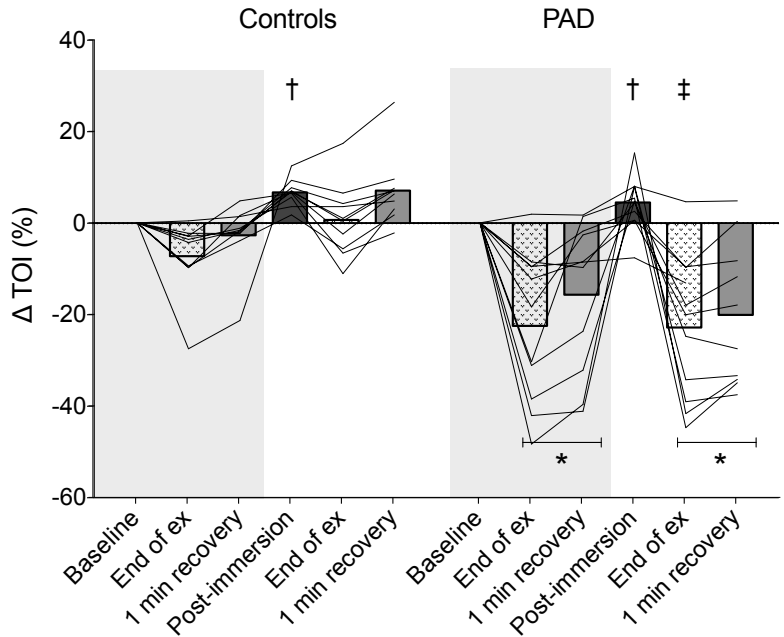

Controls
PAD
†
†
†
†
250
200
150
100
50
0
Flow (mL/min)
Baseline
Immersion
Post
Baseline
Immersion
Post


a)
Controls
PAD
1000
500
0
-500
-1000
Δ O2Hb (μM·cm)
†
†
‡
*
*
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery
b)
Controls
PAD
1000
500
0
Δ HHb (μM·cm)
*
*
‡
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery

c)


Controls
PAD
Δ nTHI
0.4
0.3
0.2
0.1
0.0
-0.1
†
*
†
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery

d)


Controls
PAD
Δ TOI (%)
40
20
0
-20
-40
-60
†
†
‡
*
*
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery
Baseline
End of ex
1 min recovery
Post-immersion
End of ex
1 min recovery